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Tetrahedron 59 (2003) 8415–8427

TETRAHEDRON

# Solid-phase synthesis of core 2 O-linked glycopeptide and its enzymatic sialylation

Yutaka Takano, Naoya Kojima, Yuko Nakahara, Hironobu Hojo and Yoshiaki Nakahara*

*Department of Applied Biochemistry, Institute of Glycotechnology, Tokai University, 1117 Kitakaname, Hiratsuka-shi, Kanagawa 259-1292, Japan*

Received 25 June 2003; accepted 19 August 2003

**Abstract**—The core 2-type tetrasaccharide building blocks (**1a/1b**) for solid-phase synthesis of glycopeptide were synthesized via stereoselective glycosylation of the disaccharyl Ser/Thr (**3a/3b**) with a glycosyl fluoride (**2**) carrying the 2-trichloroacetamido group that was readily converted into a 2-acetamido group by reduction. A segment of glycoprotein leukosialin (215–224) was synthesized by the solid-phase protocol, the building block (**1b**) being utilized. Cleavage of the synthetic glycopeptide from resin was effected with reagent K and subsequent treatment of the product with a cocktail for the 'low-acidity TfOH' facilitated complete removal of the benzyl groups with minimum loss of glycosidic linkages. To the deprotected glycopeptide (**21**), were enzymatically introduced *N*-acetylneuraminic acid (sialic acid) residues in remarkably high efficiency by using the specific sialyltransferases.
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## 1. Introduction

There is increasing demand for a variety of glycopeptide samples to be used for studying glycoprotein-mediated biological phenomena. Over the past 10 years, we have been engaged in research directed toward the establishment of facile synthetic methods for glycopeptides with unambiguous glycan structure. The usefulness of the synthesized glycopeptides in biological studies has recently been demonstrated by a chitobiose-linked henhexacontapeptide, the Ig domain I of extracellular matrix metalloproteinase inducer (Emmprin) which is a metastasis-related glycoprotein produced by tumor cells and stimulates fibroblasts to release proteolytic enzymes. The synthesized glycopeptide was proven to present remarkable matrix metalloproteinase 2 (MMP 2)-inducing activity, while little activity was observed for the non-glycosylated peptide.[1] More recently, we have accomplished synthesis of the pentasaccharide-linked Ig domain fully based on the Fmoc solid-phase protocol.[2] In addition, we have achieved the solid-phase synthesis of O-linked (core 1) glycopeptide such as the B-chain of α2HS glycoprotein,[3] the N-terminal region of glycophorin A,[4] and the C-terminal of hCG (human chorionic gonadotropin) β-subunit.[5] In these studies either benzyl-protected or non-protected oligosaccharide-linked Fmoc amino acids were utilized as the key building blocks. We also became interested in the synthesis of glycopeptide carrying a biologically significant core 2 O-linked glycan. The glycans are aberrantly expressed on the T-cell surface glycoproteins due to altered O-glycosylation both when the T-cells are activated by IL-2 and anti-CD 3 antibodies,[6] and when the T-cells are derived from patients with immunodeficiency syndromes such as Wiskott–Aldrich syndrome[7] and AIDS.[8] The typical structure of core 2 oligosaccharide, disialylated glycohexaosyl threonine, has recently been synthesized in a benzyl-protected form based on coupling of the strategically designed trisaccharide donor and acceptors.[9] However, the quantity of hexasaccharide obtained was inadequate to use further in the studies of solid-phase synthesis of a glycopeptide. Therefore, we decided to design an alternative route to the core 2 hexasaccharide by taking advantage of enzymatic sialylation. Enzymatic technologies are now readily applicable by using commercial enzymes and have often been utilized for the syntheses of sialoglycopeptides not only in solution but also on resin.[10,11] In this paper, we describe the stereocontrolled synthesis of core 2 tetrasaccharide building blocks, solid-phase synthesis of a glycopeptide with the building block, and sialylation of the glycopeptide with the commercial α2-3 sialyltransfases. An outline of this study is depicted in Figure 1. The early part of this work has preliminarily been reported.[12]

## 2. Synthesis of the building block 1a and 1b

Synthetic studies were started for the key building blocks **1a** and **1b**. Based on consideration of convergent synthesis strategy, the tetrasaccharide was retrosynthesized into two

*Keywords*: glycosylation; glycopeptide; solid-phase synthesis; sialyltransferase.

* Corresponding author. Tel./fax: +81-463-50-2075; e-mail: yonak@keyaki.cc.u-tokai.ac.jp

0040–4020/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.tet.2003.08.047

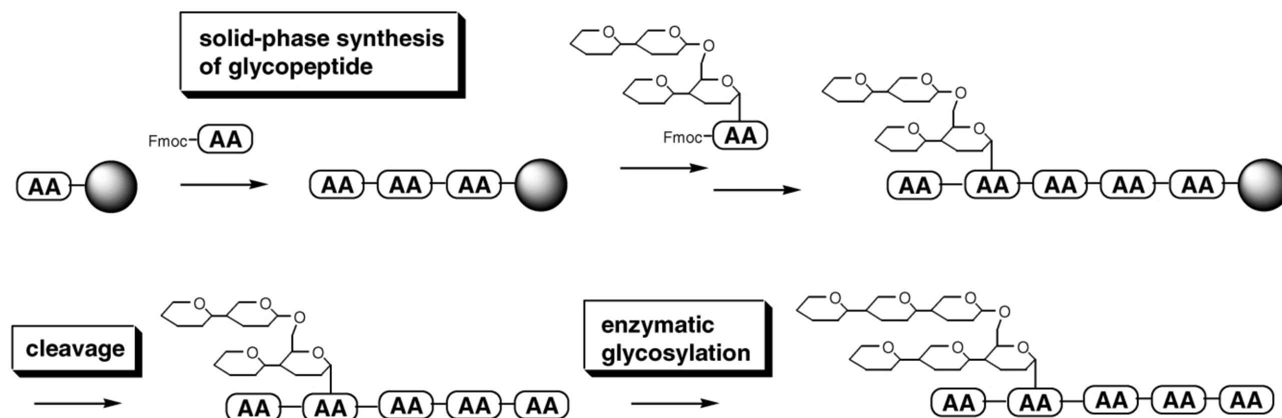



**Figure 1.** Synthetic strategy for the core 2 hexasaccharide-linked glycopeptide.

disaccharide precursors (**2** and **3**). Since disaccharyl diol **3a** and **3b** were readily produced by debenzylidenation of **4a** and **4b** which had already been prepared in this group,[13] another disaccharide **2** was synthesized as the glycosyl donor in which an *N*-trichloroacetyl protecting group was employed in order to facilitate β-selective glycosylation (Fig. 2).[14] As shown in Scheme 1, known *t*-butyldiphenylsilyl 2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside **6**[9b] was glycosylated with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide **5** in the presence of AgOTf to give lactosamine derivative **8**. Compound **8** was deacetylated with NaOMe/MeOH and then benzylidenated with benzaldehyde dimethyl acetal and *p*-TsOH to afford **10**. Protection of the resulting hydroxyl groups by benzylation and subsequent reduction of the azide group with Zn/AcOH gave amine **14**. Alternatively, compound **14** was also synthesized from known *N*-phthaloylated derivative **9**.[15] After deacetylation of **9**, benzylidenation was followed by benzylation to afford **13**, which was dephthaloylated with ethylenediamine to give **14**. Compound **14** was acylated with trichloroacetyl chloride in pyridine, and then the silyl group was removed under the conditions of fluoridolysis. The resulting hemiacetal (**16**) was converted into glycosyl fluoride **2**. The α-fluoride was produced in high stereoselectivity. Glycosylation of **3a** and **3b** with **2** was promoted by Suzuki's condition[16] to exclusively produce the desired tetrasaccharide **17a** and **17b** in 72 and 70%, respectively. Transformation of trichloroacetamide into acetamide was realized by reduction with Zn–AcOH, with accompanying reduction of the azide group. The products were acetylated with $Ac_2O$ in MeOH. Despite the sluggish dechlorination, compounds **18a** and **18b** were obtained in high yields. Allyl ester was then cleaved by catalysis of Pd(0) in the presence of dimedone as a nucleophile in THF to furnish **1a** and **1b**.

## 3. Solid-phase synthesis of glycopeptide and deprotection

With the suitably protected building block (**1b**) in hand, solid-phase synthesis of a glycopeptide carrying core 2 tetrasaccharide was next investigated. Human leukosialin (CD 43) is a T-lymphocyte transmembrane glycoprotein in which an extracellular domain is enriched with serine and threonine residues, and is heavily *O*-glycosylated. As described above, activated T-cells carry a leukosialin expressing the core 2 *O*-hexasaccharide. A segment of leukosialin (215–224) was synthesized as a model of core 2 glycopeptide, where **1b** was incorporated as the building block for $Thr^{216}$. Solid-phase synthesis was performed on the commercial Fmoc Sieber amide resin (0.25 mmol/g) according to the Fmoc procedure.[17] The resin can release the synthesized peptide carboxamides on exposure to a weakly acidic medium (e.g. 1–2% TFA in $CH_2Cl_2$). All the solid-phase reactions were manually operated in a polypropylene tube. Peptide condensation was facilitated by using excess Fmoc amino acid (4 equiv.) activated with dicyclohexylcarbodiimide (DCC), 1-hydroxybenzotriazole (HOBt), and diisopropylethylamine (DIEA) in *N*-methylpyrrolidone

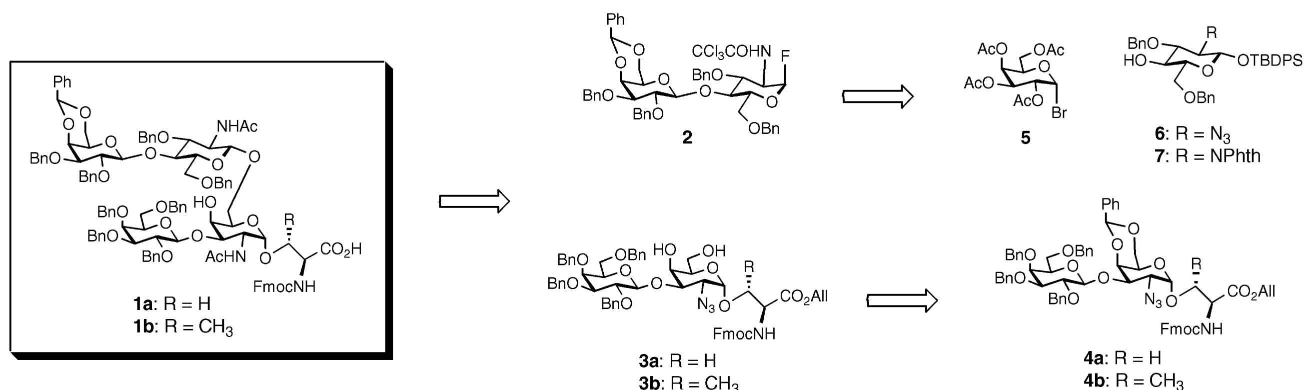
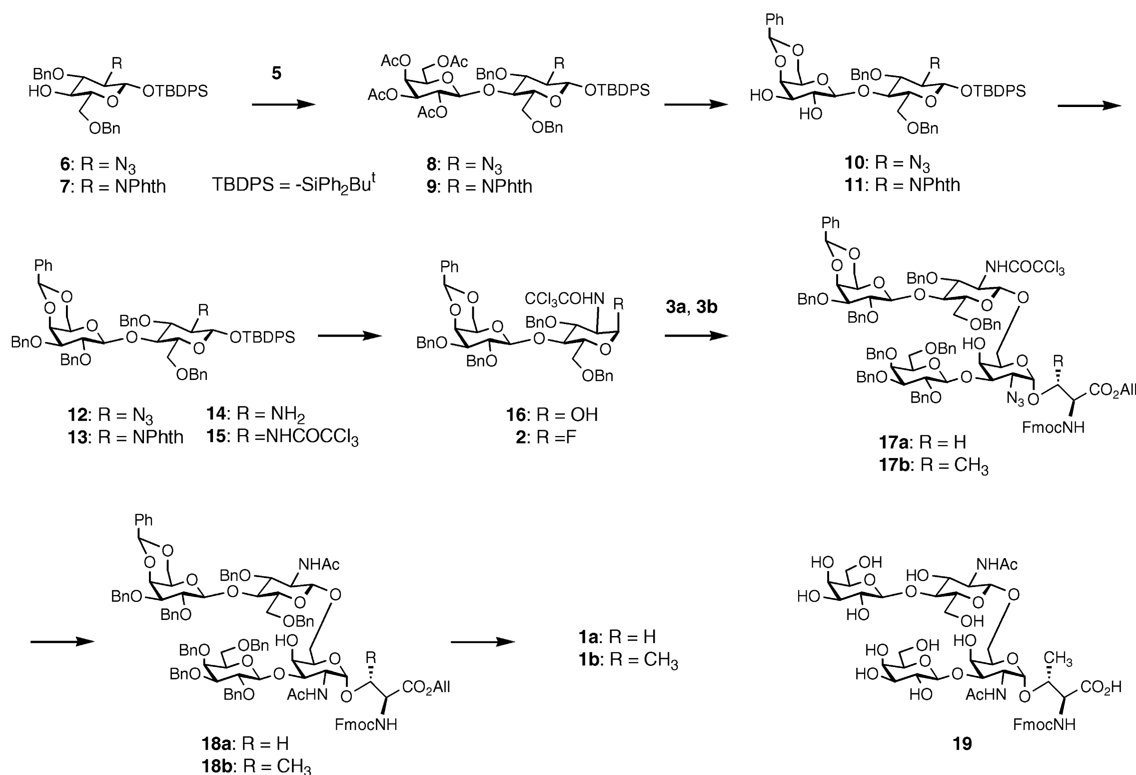
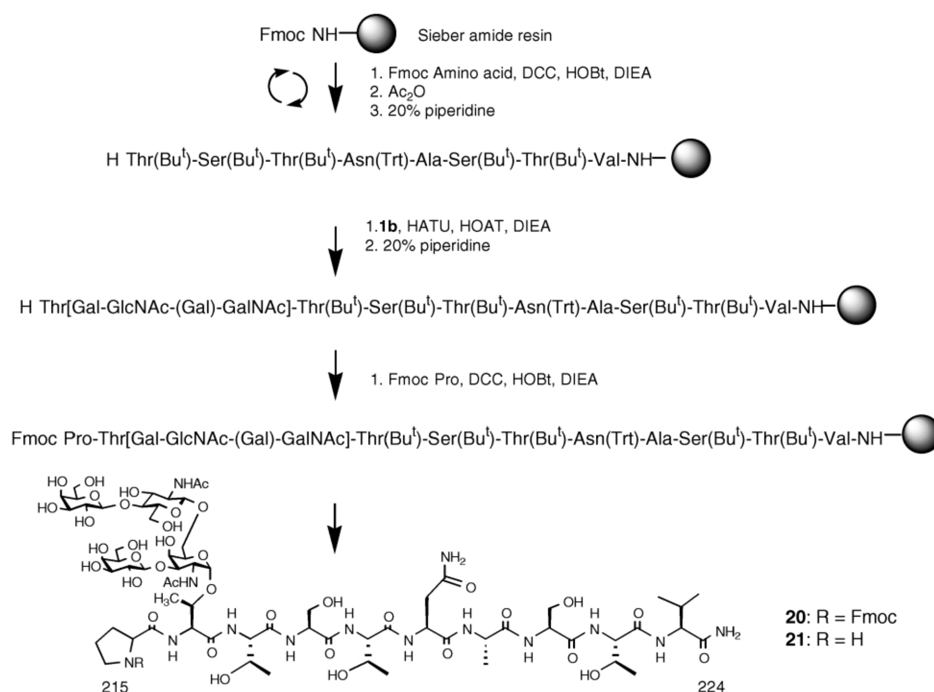



**Figure 2.** Retrosynthetic scheme of the core 2 tetrasaccharide buildimg blocks **1a** and **1b**.




**Scheme 1.** Synthesis of the tetrasaccharide building blocks **1a** and **1b**.

(NMP), whereas *N*-deprotection was effected with 20% piperidine in NMP. Side-chain functional groups of the serine/threonine and asparagine residues were protected with *t*-butyl and trityl groups, respectively. The unreacted amino groups on the resin were capped with $Ac_2O$ at the end of each peptide-coupling procedure until introduction of the hydroxyl group-containing tetrasaccharide (**1b**). The octapeptide (217–224) assembled on the resin was *N*-deprotected and allowed to react with 2 equiv. of **1b** in the presence of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) in NMP. The crucial coupling step was run overnight. The coupling efficiency seemed comparable with that of the reaction run under activation with the standard DCC–HOBt combination. The resulting resin was *N*-deprotected and condensed with Fmoc-Pro–OH to complete the desired




**Scheme 2.** Solid-phase synthesis of the core 2 tetrasaccharide-linked peptide **21**.

sequence. Conditions for deprotection of the carbohydrate moiety were next explored with tetrasaccharide **1b**, before the synthesized glycopeptide was released from the resin. Among the procedures tested, the 'low-acidity TfOH' method developed for debenzylation of the synthetic peptides was the most promising.[18] Compound **1b** was treated with TfOH in a mixture of dimethylsulfide (DMS), *m*-cresol, and trifluoroacetic acid (TFA) at −15°C for 1 h. The reaction was quenched with ethereal pyridine and the products were analyzed by HPLC and MALDI TOF MS. Figure 3(a) shows the chromatographic profile of the products. The largest peak (1) represents the desired tetrasaccharide (**19**), which comprises about 88% of the carbohydrate-derived components. The small peaks 2 and 3 coincided in the mass spectra with the compounds lacking one of the galactose residues, while peak 4 corresponded to the disaccharide-linked threonine derived from scission of the β-GlcNAc glycosidic linkage. Mass spectra of the peaks

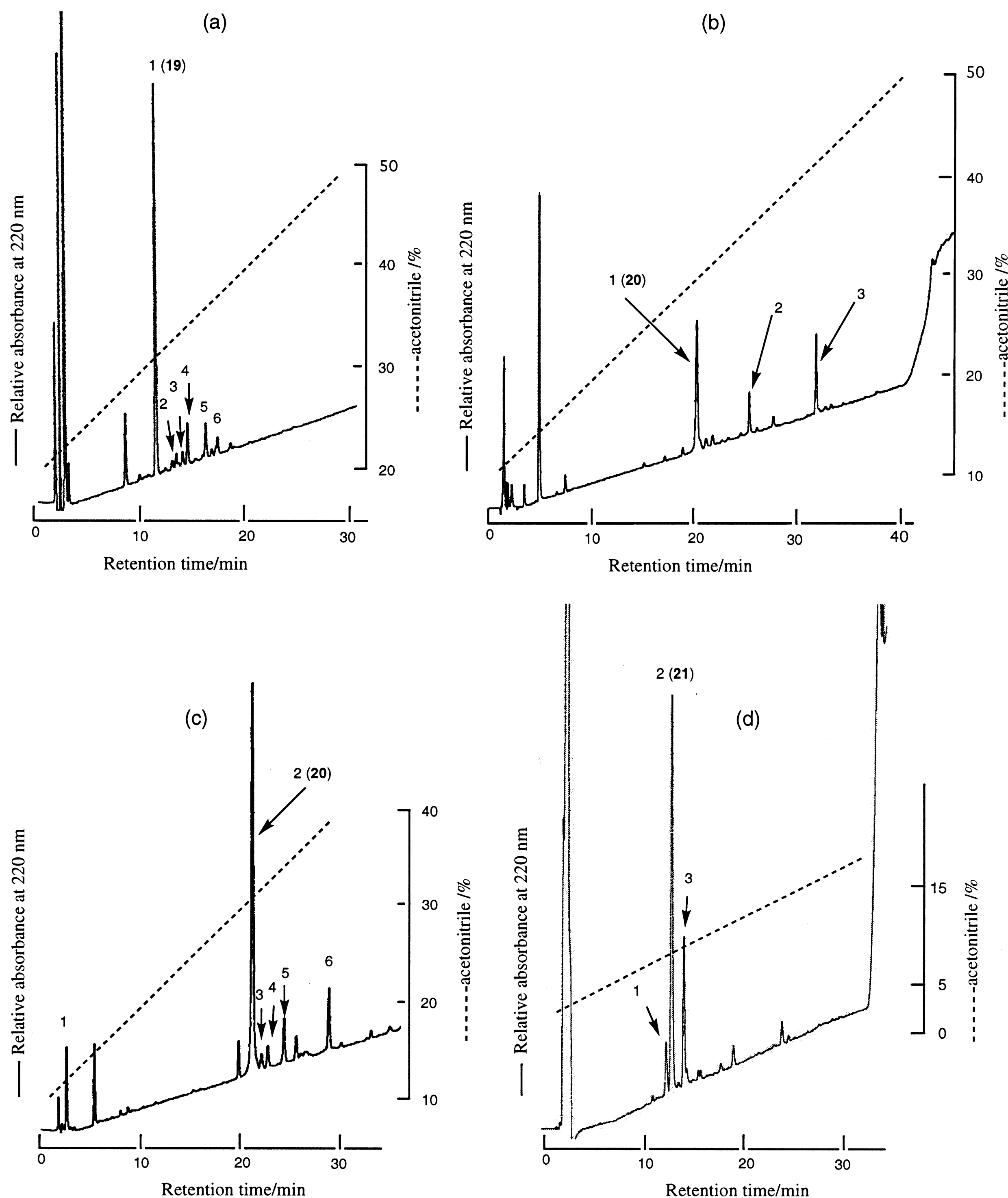



**Figure 3.** HPLC of the debenzylation products of **1b** (a), cleavage of the glycopeptide from the resin under the low-acidity TfOH conditions (b), the debenzylation products obtained through reagent K treatment (c), and the fully deprotected glycopeptide **21** (d). Column: Mightysil RP-18 (150×4.6 mm). Eluent A: distilled water containing 0.1% TFA, B: acetonitrile containing 0.1% TFA. Flow rate: 1 ml/min.

in the less mobile fraction (5–6) agreed with those of the compounds carrying one benzyl group. The deprotection reaction performed at higher temperature (0°C) gave rise to apparent increases in the scission products. Based on these experiments, we then moved to the deprotection studies with the synthesized glycopeptide. The resin was treated with the cocktail for the low-acidity TfOH for 1 h at −15°C and the products precipitated by ethereal pyridine were analyzed in the same manner. An HPLC profile of the products is shown in Figure 3(b). Three major products were separated and characterized by mass spectrometry. The desired glycopeptide [**20**: $(M+Na)^+$: 1951.45] was eluted in the fraction at the retention time of 22 min. The mass spectrum of the second product showed the molecular ion for the glycothreonine-missing product (octapeptide) with a Trt group unremoved [$(M+Na)^+$: 1042.97]. The third product exhibited the molecular ion $(M+Na)^+$ of 2193.57 corresponding to the glycopeptide carrying a Trt group. Therefore, we concluded that the procedure was insufficient to cleave the *N*-Trt linkage. To achieve complete removal of the Trt group, separate procedures for cleavage from resin and for debenzylation were employed. The resin was treated with reagent K (aq. $CF_3CO_2H$, thioanisole, 1,2-ethanedithiol, phenol) for 1 h at ambient temperature. The product and the resin were precipitated from ether and subjected to the debenzylation under the conditions of low-acidity TfOH for 1 h at −15°C. The products were analyzed in detail, and it was found that the desired glycopeptide (**20**) was accompanied with the incompletely debenzylated glycopeptides (5–7%). Consequently, the debenzylation procedure was performed for 2 h. Figure 3(c) exhibits an HPLC profile of the mixture of products. In the chromatogram, peaks 1–6 are derived from (glyco)peptides. Peak 2 corresponds to the tetrasaccharyl decapeptide (**20**) [$(M+Na)^+$: 1951.31], while the highly hydrophilic component (peak 1) is the unreacted octapeptide [$(M+H)^+$: 778.62]. Peaks 3 [$(M+Na)^+$: 1789.73] and 4 [$(M+Na)^+$: 1789.68] are the glycopeptides losing a Gal residue. Peak 5 [$(M+Na)^+$: 1586.77] is attributable to the glycopeptide lacking a Gal·GlcNAc residue. The peptide skipping the glycothreonine unit appears as peak 6 [$(M+Na)^+$: 1120.83]. In a similar manner, the fully deprotected glycopeptide (**21**) was produced as follows. The tetrasaccharide-linked glycopeptide on resin was *N*-deprotected with 20% piperidine, cleaved with reagent K, and debenzylated with the mixture of diluted TfOH. An HPLC profile of the products is shown in Figure 3(d). Three peaks are observed, in which the largest one [$(M+H)^+$: 1707.89, $(M+Na)^+$: 1729.65] represents **21**. Peak 1 corresponds to the unreacted octapeptide, whereas the glycopeptide lacking a Gal residue is included in the fraction of peak 3. The isolation of glycopeptide **21** was performed by preparative HPLC and the overall yield was 27% (Scheme 2).

## 4. Enzymatic sialylation of the glycopeptide

Enzymatic sialylation of the synthesized glycopeptide was next investigated. The neccessary enzymes, β-Gal-β1,3/4-GlcNAc-α2,3-sialyltransferase and β-Gal-β1,3-GalNAc-α2,3-sialyltransferase, are commercially available. The former sialyltransferase promotes formation of an α-Neu5Ac-(2→3)-β-Gal-(1→4)-GlcNAc oligosaccharide, and the latter specifically gives an α-Neu5Ac-(2→3)-β-Gal-(1→3)-GalNAc motif. In order to examine the efficiency of the enzymatic reactions with these sialyltransferases, tetrasaccharide **19** was enzymatically sialylated (Fig. 4). A mixture of substrate **19** and CMP–sialic acid (5 equiv.) was incubated with the recombinant rat β-Gal-β1,3/4-GlcNAc-α2,3-sialyltransferase, BSA and

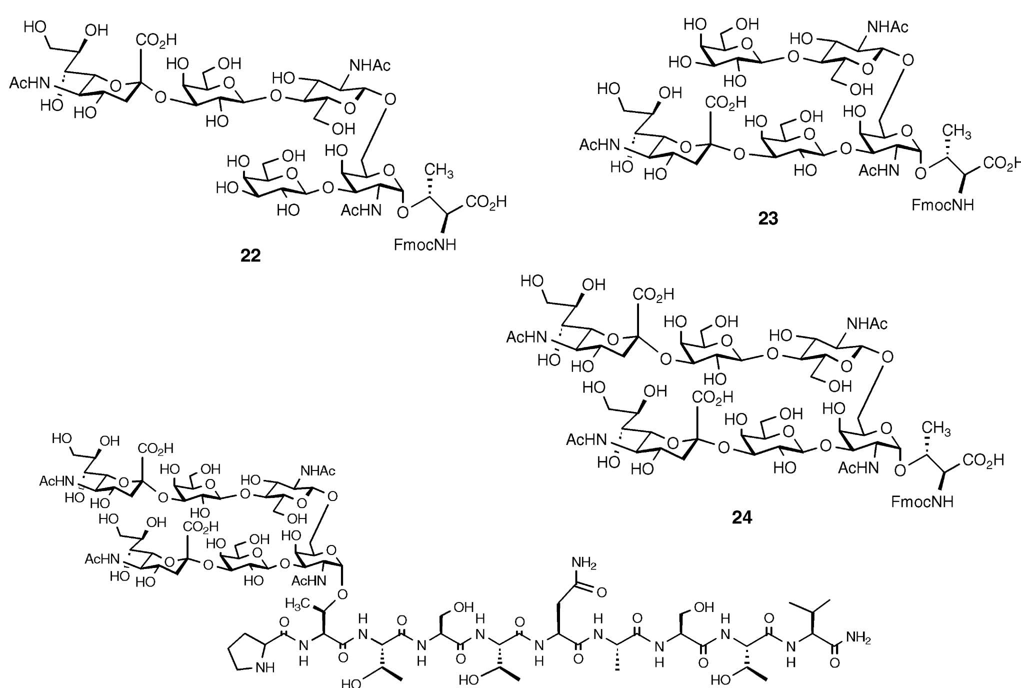



**Figure 4.** Structures of the enzymatic sialylation products.

MOPS buffer (pH 7.4) at 37°C. The products were analyzed by HPLC and MALDI TOF MS. Figure 5(a) shows a chromatogram of the reaction mixture of 24 h incubation, where three new compounds are observed as peaks 1–3, and unconverted **19** is detected as peak 4. Peak 1 (15%) was identified as disialylated product **24** [$(M+Na)^+$: 1676.86], whereas the mass spectra of peak 2 (8%) and 3 (57%) demonstrated the molecular ions for monosialocompounds, **23** (1385.86) and **22** (1385.83), respectively. When the incubation was extended to 48 h, reversed reaction took place to afford a mixture of **24** (9%), **23** (13%), **22** (41%), and **19** (37%). Further elongation of the incubation time to 72 h led to an increase in regenerated **19** (49%). On the other hand, the recombinant rat β-Gal-β1,3-GalNAc-α2,3-sialyltransferase displayed high specificity in the sialylation of **19**. The enzymatic reaction was carried out at pH 6.0 for 16 h. HPLC analysis of the product is shown in Figure 5(b), where the specifically sialylated product (**23**: 86%) is observed. The longer incubation again resulted in the reversed reaction. Subsequently, we carried out the concurrent sialy transfer reaction using two enzymes and excess CMP–sialic acid (7.5 equiv. for each sialylation) at pH 6.8. The results are shown in Figure 5(c). The desired disialylation was predominant (**24**: 90%) in the mixture of 12 h incubation. The monosialo derivatives (**23**: 4% and **22**: 6%) were also detected.

On the basis of these results, we next examined the sialylation of glycopeptide **21**. The glycopeptide (100 nmol) was incubated with two enzymes and CMP–sialic acid for 12 h under the same conditions as tested for concurrent sialylation of **19**. As shown in Figure 6, the disialylation was remarkably successful to exclusively afford hexasaccharide-linked peptide **25** (M+Na: 2311.35). In the upper half of the figure is given a reference chromatogram derived from a purposely prepared mixture of **21** (peak 1), the individually prepared monosialoglycopeptides **26**, **27** (peaks 2 and 3), and **25** (peak 4). A 10 mM ammonium acetate buffer (pH 5.8) used as the eluent with acetonitrile was effective to separate the glycopeptides on the C-18 reversed phase column, while a combination of 0.1% TFA-containing water and acetonitrile eluted all the glycopeptides, 19, 25, 26 and 27, as the indistinguishable peaks on the same column. The disialylated product 25 was the least mobile in the weakly acidic eluent. It has not been known whether this unusual chromatographic behavior depends upon the property of the ionized sialic acid residues or the altered conformation of glycopeptide in this medium. As this experiment demonstrated that the concurrent sialylation proceeded in a highly efficient manner, a variety of core 2 sialoglycopeptides will be synthesized by applying the simple procedure even in a larger scale.

In summary, the highly stereoselective formation of β-GlcNAc-(1→6)-GalNAc linkage of the core 2 O-linked glycan was accomplished by employing 2-trichloroacetamide as the stereocontrolling group. The tetrasaccharide-linked threonine thus prepared in a benzyl-protected form was used for solid-phase synthesis of the mucin type domain of leukosialin (215–224). Deprotection was efficiently

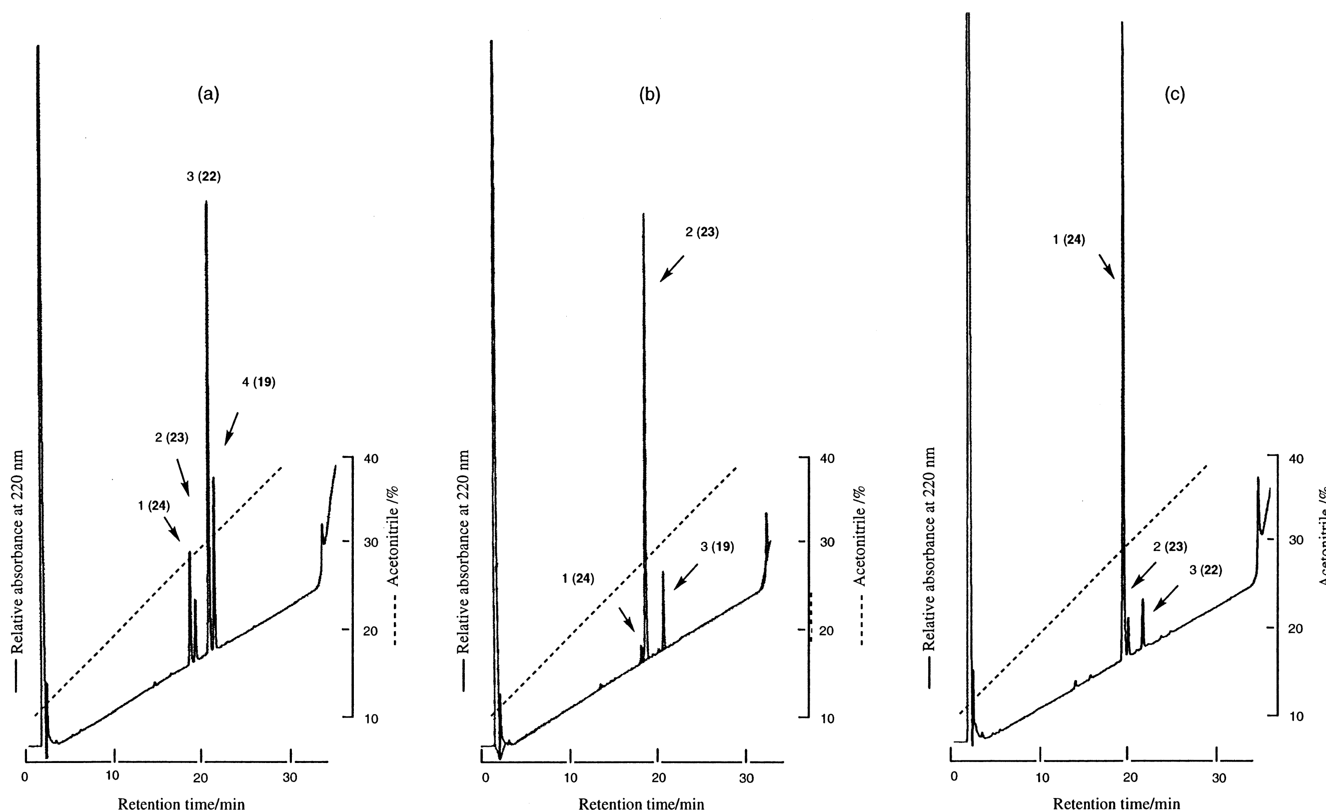



**Figure 5.** HPLC of the sialylation products of **19** with β-Gal-β1,3/4-GlcNAc-α2,3-sialyltransferase (a) the sialylation products of **19** with β-Gal-β1,3-GalNAc-α2,3-sialyltransferase (b) and the sialylation products of **19** with β-Gal-β1,3/4-GlcNAc-α2,3-sialyltransferase and β-Gal-β1,3-GalNAc-α2,3-sialyltransferase (c) Column: Mightysil RP-18 (150×4.6 mm). Eluent A: distilled water containing 0.1% TFA, B: acetonitrile containing 0.1% TFA. Flow rate: 1 ml/min.

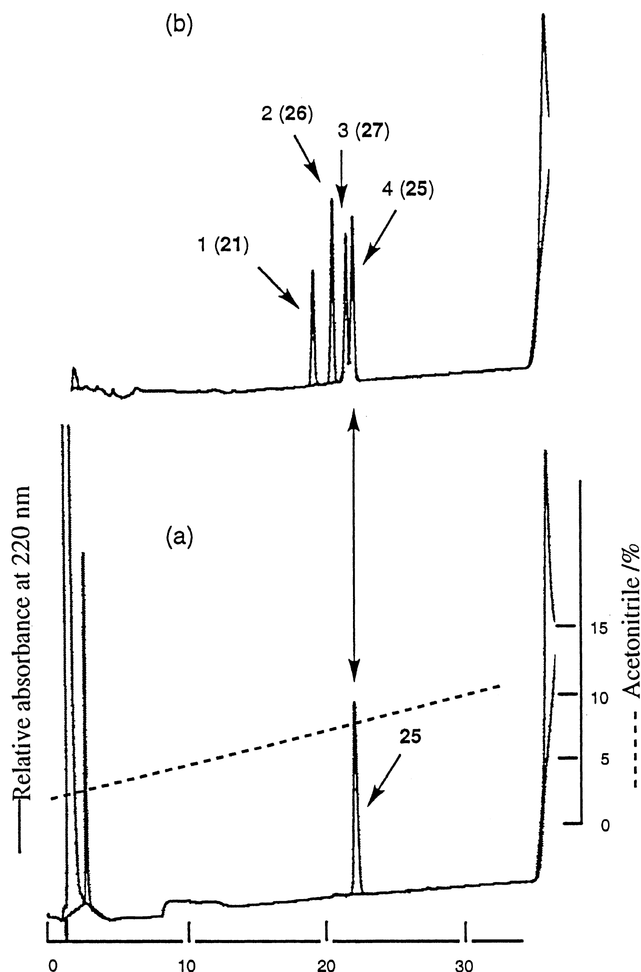



**Figure 6.** HPLC of the reaction mixture of the enzymatic sialylation of **21** (a) The upper chromatogram (b) shows a reference mixture of **21**, monosialoglycopeptides, and **25**. Column: Mightysil RP-18 (150×4.6 mm). Eluent A: 10 mM ammonium acetate buffer (pH 5.8), B: acetonitrile containing 10% eluent A. Flow rate: 1 ml/min.

achieved by utilizing the low-acidity TfOH conditions. The resulting glycopeptide was subjected to enzymatic glycosylation, being used as the substrate for the sialyltransferases. The desired hexasaccharide-linked glycopeptide was successfully produced in high efficiency. Further efforts to extend these results to synthesize glycopeptides with the clustered oligosaccharides are currently underway in this laboratory.

## 5. Experimental

### 5.1. General

Optical rotations were determined with a Jasco DIP-370 polarimeter for solutions in $CHCl_3$, unless noted otherwise. Column chromatography was performed on silica gel PSQ 100B (Fuji Silysia). TLC and HPTLC were performed on Silica Gel 60 $F_{254}$ (E. Merck). $^1$H and $^{13}$C NMR spectra were recorded with a Jeol AL400 [$^1$H (400 MHz), $^{13}$C (100 MHz)] spectrometer. Chemical shifts are expressed in ppm downfield from the signal for internal $Me_4Si$ for solutions in $CDCl_3$. MALDI TOF mass spectra were obtained with a PerSeptive Voyager-DE PRO spectrometer (2,5-dihydroxybenzoic acid was used as a matrix). High resolution Fab mass spectra were measured with JEOL JMS HX-110 spectrometer (2,5-dihydroxybenzoic acid was used as a matrix). All solid-phase reactions were performed at room temperature in the capped polypropylene test tubes with stirring on a vortex tube-mixer. HPLC was performed using Mightysil RP-8, RP-18 (150×4.6 mm for analysis and 250×10 mm for preparation, Kanto Chemical Co.). Amino acids were analyzed on a Hitachi L-8500 amino acid analyzer. Fmoc Sieber amide MBHA resin was purchased from NOVA Biochem. Sialyltransferases [α2,3-(*N*)-sialyltransferase, rat, recombinant, and α2,3-(*O*)-sialyltransferase, rat, recombinant] and CMP–sialic acid were purchased from CALBIOCHEM.

**5.1.1. *N*-(9-Fluorenylmethoxycarbonyl)-*O*-[2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-α-D-galactopyranosyl]-L-serine allyl ester 3a.** A mixture of **4a** (494 mg, 0.43 mmol) in $CH_2Cl_2$ (10 ml) and 80% aq. TFA (4 ml) was stirred at −15°C for 1 h, then neutralized with sat. $NaHCO_3$, and extracted with $CHCl_3$. The extract was successively washed with water, sat. $NaHCO_3$, and brine, dried over $Na_2SO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel with toluene–EtOAc (1:1) to afford **3a** (340 mg, 74%). $[\alpha]_D$=+67.2° (*c* 1). $R_f$ 0.37 (1:1 toluene–EtOAc). $^1$H NMR: δ 7.75 (d, 2H, *J*=7.3 Hz, Ar), 7.63–7.56 (m, 2H, Ar), 7.53–7.31 (m, 4H, Ar), 7.30–7.10 (m, 20H, Ar), 6.04 (d, 1H, *J*=8.3 Hz, N*H*), 5.90 (m, 1H, $-CH_2CH{=}CH_2$), 5.32 (brd, 1H, *J*=17.1 Hz, $-CH_2{=}CH_2$), 5.24 (brd, 1H, *J*=10.5 Hz, $-CH_2{=}CH_2$), 4.94 (d, 1H, *J*=3.2 Hz, H-1a), 4.52 (d, 1H, *J*=7.8 Hz, H-1b). MALDI TOF MS: calcd for $C_{61}H_{64}N_4O_{14}\cdot Na$ *m/z* 1099.43. Found 1099.04. Anal. calcd for $C_{61}H_{64}N_4O_{14}$: C, 68.02; H, 5.99; N, 5.20. Found: C, 67.75; H, 6.02; N, 5.09.

**5.1.2. *N*-(9-Fluorenylmethoxycarbonyl)-*O*-[2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→3)-2-azido-2-deoxy-α-D-galactopyranosyl]-L-threonine allyl ester 3b.** Compound **4b** (178 mg, 0.15 mmol) was debenzylidenated as described above. Chromatography of the crude product on silica gel with toluene–EtOAc (4:1) afforded **3b** (138 mg, 84%). $[\alpha]_D$=+60.8° (*c* 1). $R_f$ 0.15 (4:1 toluene–EtOAc). $^1$H NMR: δ 7.75 (d, 2H, *J*=7.3 Hz, Ar), 7.60 (m, 2H, Ar), 7.41–7.24 (m, 24H, Ar), 5.92 (m, 1H, $-CH_2CH{=}CH_2$), 5.71 (d, 1H, *J*=9.5 Hz, N*H*), 5.34 (brd, 1H, *J*=17.1 Hz, $-CH_2{=}CH_2$), 5.24 (brd, 1H, *J*=10.5 Hz, $-CH_2{=}CH_2$), 5.05 (d, 1H, *J*=3.7 Hz, H-1a), 4.52 (d, 1H, *J*=7.8 Hz, H-1b), 1.32 (d, 1H, *J*=6.3 Hz, Thr γH). MALDI TOF MS: calcd for $C_{62}H_{66}N_4O_{14}\cdot Na$ *m/z* 1113.45. Found 1113.46.

Anal. calcd for $C_{62}H_{66}N_4O_{14}$: C, 68.24; H, 6.10. Found: C, 68.13; H, 6.16.

**5.1.3. *tert*-Butyldiphenylsilyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside 8.** To a stirred mixture of **6** (2.13 g, 3.42 mmol), AgOTf (3.52 g, 13.7 mmol), and dried molecular sieves 4 Å powder (20 g) in anhydrous $ClCH_2CH_2Cl$ (40 ml) was added a solution of **5** (1.69 g, 4.1 mmol) in $ClCH_2CH_2Cl$ (20 ml) at −15°C under Ar. The mixture was stirred at the temperature for 3.5 h before the reaction was quenched with pyridine and water. The mixture was filtered through Celite, and the filtrate was

extracted with $CHCl_3$. The extract was successively washed with water, sat. $NaHCO_3$, and brine, dried over $Na_2SO_4$, and concentrated in vacuo. The crude product was chromatographed on silica gel with hexane–EtOAc (2:1) to give **8** (2.59 g, 79%). $[\alpha]_D=-22.1°$ (*c* 1). $R_f$ 0.47 (1:1 hexane–EtOAc). $^1$H NMR: $\delta$ 7.74–7.69 (m, 20H, Ar), 5.26 (brd, 1H, H-4b), 5.08 (dd, 1H, $J=8.1$, 10.3 Hz, H-2b), 4.93 (d, 1H, $J=11.0$ Hz, $-CH_2Ph$), 4.84 (dd, 1H, $J=3.4$, 10.3 Hz, H-3b), 4.72 (d, 1H, $J=10.5$ Hz, $-CH_2Ph$), 4.61 (d, 1H, $J=8.1$ Hz, H-1b), 4.55 (d, 1H, $J=12.0$ Hz, $-CH_2Ph$), 4.33 (d, 1H, $J=$ 7.8 Hz, H-1a), 4.27 (d, 1H, $J=12.0$ Hz, $-CH_2Ph$), 4.03–3.97 (m, 2H, H-4a, H-6b), 3.84 (dd, 1H, $J=5.9$, 11.2 Hz, H-6b), 3.61 (m, 1H, H-5b), 3.54–3.45 (m, 2H, H-2a, H-6a), 3.29–3.24 (m, 2H, H-3a, H-6a), 2.85 (m, 1H, H-5a), 2.09, 1.98, 1.95, and 1.90 (4s, 12H, 4 Ac), 1.11 (s, 9H, *t*-Bu). MALDI TOF MS: calcd for $C_{50}H_{59}N_3O_{14}Si\cdot Na$ *m/z* 976.17. Found 975.96. Anal. calcd for $C_{50}H_{59}N_3O_{14}Si$: C, 62.94; H, 6.23; N, 4.40. Found: C, 62.91; H, 6.27; N, 4.19.

**5.1.4. *tert*-Butyldiphenylsilyl 4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside 10.** A solution of **8** (1.06 g, 1.11 mmol) in MeOH (25 ml) was stirred with 1 M NaOMe/MeOH (0.56 ml, 0.56 mmol) at room temperature for 1 h. The mixture was neutralized with Amberlist 15 and filtered. The filtrate was concentrated in vacuo to the residue, which was dissolved in anhydrous $CH_3CN$ (30 ml) and stirred with benzaldehyde dimethyl acetal (0.48 ml, 3.32 mmol) and a catalytic amount of *p*-TsOH at room temperature for 1 h. The reaction was quenched by an addition of sat. $NaHCO_3$, and the mixture was concentrated in vacuo. The residue was extracted with ether. The extract was successively washed with sat. $NaHCO_3$, water, and brine, dried over $Na_2SO_4$, and concentrated in vacuo. The crude product was chromatographed on silica gel with hexane–EtOAc (1:1) to afford **10** (0.87 g, 88%). $[\alpha]_D=-23.7°$ (*c* 1). $R_f$ 0.49 (EtOAc). $^1$H NMR: $\delta$ 7.72–7.68 and 7.44–7.16 (m, 25H, Ar), 5.49 [s, 1H, Ph$CH$(O–)$_2$], 5.00 (d, 1H, $J=11.0$ Hz, $-CH_2Ph$), 4.86 (d, 1H, $J=11.0$ Hz, $-CH_2Ph$), 4.52 (d, 1H, $J=7.8$ Hz, H-1b), 4.51 (d, 1H, $J=12.2$ Hz, $-CH_2Ph$), 4.35 (d, 1H, $J=$ 7.8 Hz, H-1a), 4.32 (d, 1H, $J=12.2$ Hz, $-CH_2Ph$), 4.12–4.00 (m, 3H, H-4a, H-4b, H-6a), 3.80–3.76 (m, 2H, H-6a, H-6b), 3.73 (t, 1H, $J=7.8$ Hz, H-2b), 3.52–3.46 (m, 2H, H-2a, H-3b), 3.35 (t, 1H, $J=9.7$ Hz, H-3a), 3.27 (br, 1H, H-6b), 2.95–2.93 (m, 2H, H-5a, H-5b), 1.12 (s, 9H, *t*-Bu). MALDI TOF MS: calcd for $C_{49}H_{55}N_3O_{10}Si\cdot Na$ *m/z* 896.36. Found 896.52. Anal. calcd for $C_{49}H_{55}N_3O_{10}Si$: C, 67.33; H, 6.34; N, 4.81. Found: C, 67.41; H, 6.38; N, 4.77.

**5.1.5. *tert*-Butyldiphenylsilyl 4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside 11.** Compound **9** (6.33 g, 5.98 mmol) was deacetylated with NaOMe in MeOH, and benzylidenated with benzaldehyde dimethyl acetal (2.6 ml, 18.0 mmol) and *p*-TsOH in anhydrous $CH_3CN$ 150 ml) as described for **10**. The product was chromatographed on silica gel with toluene–EtOAc (1:1) to give **11** (4.70 g, 97%). $[\alpha]_D=+12.8°$ (*c* 1). $R_f$ 0.41 (1:1 toluene–EtOAc). $^1$H NMR: $\delta$ 7.69–7.59, 7.41–7.16, and 7.07–6.84 (m, 25H, Ar), 5.45 [s, 1H, Ph$CH$(O–)$_2$], 5.19 (d, 1H, $J=7.6$ Hz, H-1a), 4.92 (d, 1H, $J=12.4$ Hz, $-CH_2Ph$), 4.64 (d, 1H, $J=12.4$ Hz, $-CH_2Ph$), 4.60 (d, 1H, $J=7.6$ Hz, H-1b), 4.52 (d, 1H, $J=12.4$ Hz, $-CH_2Ph$), 4.46 (d, 1H, $J=12.4$ Hz, $-CH_2Ph$), 4.08 (d, 1H, $J=3.6$ Hz, H-4b), 3.66 (brt, 1H, H-2b), 1.62 (s, 9H, *t*-Bu). Anal. calcd for $C_{57}H_{59}NO_{12}Si$: C, 69.99; H, 6.08; N, 1.43. Found: C, 69.80; H, 6.10; N, 1.37.

**5.1.6. *tert*-Butyldiphenylsilyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside 12.** A mixture of **10** (370 mg, 0.42 mmol) and 60% NaH (68 mg, 1.70 mmol) in anhydrous THF (70 ml) was heated at 60°C with stirring for 1 h. To the mixture was added benzyl bromide (0.29 ml, 1.69 mmol). The resulting mixture was heated overnight at the temperature. After cooling, a piece of ice was carefully added to the mixture. The mixture was concentrated in vacuo to the residue, which was extracted with ether–EtOAc (1:1). The extract was successively washed with water and brine, dried over $Na_2SO_4$, and concentrated in vacuo. The crude product was chromatographed on silica gel with hexane–EtOAc (4:1) to give **12** (405 mg, 91%). $[\alpha]_D=-6.4°$ (*c* 1). $R_f$ 0.10 (4:1 hexane–EtOAc). $^1$H NMR: $\delta$ 7.72–7.70 and 7.53–7.08 (m, 35H, Ar), 5.46 [s, 1H, Ph$CH$(O–)$_2$], 5.18 (d, 1H, $J=10.5$ Hz, $-CH_2Ph$), 4.76 (d, 1H, $J=10.9$ Hz, $-CH_2Ph$), 4.71 (brs, 2H, $-CH_2Ph$), 4.69 (d, 1H, $J=11.2$ Hz, $-CH_2Ph$), 4.62 (d, 1H, $J=10.9$ Hz, $-CH_2Ph$), 4.45 (d, 1H, $J=7.8$ Hz, H-1b), 4.37 (d, 1H, $J=$ 11.9 Hz, $-CH_2Ph$), 4.31 (d, 1H, $J=8.1$ Hz, H-1b), 4.21 (brd, 1H, $J=11.2$ Hz, H-6b), 4.16 (d, 1H, $J=11.9$ Hz, $-CH_2Ph$), 4.04 (d, 1H, $J=3.8$ Hz, H-4b), 4.00 (t, 1H, $J=8.1$ Hz, H-4a), 3.88 (dd, 1H, $J=1.7$, 12.2 Hz, H-6b), 3.74–3.69 (m, 2H, H-2b, H-6a), 3.46 (dd, 1H, $J=7.8$, 9.8 Hz, H-2a), 3.41 (dd, 1H, $J=3.7$, 7.8 Hz, H-3b), 3.27 (t, 1H, $J=8.8$ Hz, H-3a), 3.18 (brd, 1H, $J=10.0$ Hz, H-6a), 3.05 (brs, 1H, H-5b), 2.78 (brd, 1H, H-5a), 1.10 (s, 9H, *t*-Bu). MALDI TOF MS: calcd for $C_{63}H_{67}N_3O_{10}Si\cdot Na$ *m/z* 1076.45. Found 1076.43. Anal. calcd for $C_{63}H_{67}N_3O_{11}Si$: C, 71.77; H, 6.41; N, 3.99. Found: C, 71.61; H, 6.38; N, 3.80.

**5.1.7. *tert*-Butyldiphenylsilyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside 13.** A mixture of **11** (694 mg, 0.70 mmol) and 60% NaH (85 mg, 2.1 mmol) in anhydrous DMF (15 ml) was stirred at 0°C under Ar for 15 min. Then benzyl bromide (0.25 ml, 2.1 mmol) was added to the mixture, which was stirred overnight at room temperature. The reaction was quenched by addition of ice–$NH_4Cl$ aq. and the mixture was concentrated in vacuo. The product was extracted with ether–EtOAc (1:1), washed successively with water and brine, and dried over $Na_2SO_4$. Concentration of the extract in vacuo gave the crude product, which was chromatographed on silica gel with toluene–EtOAc (9:1) to give **13** (545 mg, 66%). $[\alpha]_D=+12.3°$ (*c* 1). $R_f$ 0.18 (9:1 toluene–EtOAc). $^1$H NMR: $\delta$ 7.39–7.17, 7.13–6.97, and 6.80–6.73 (m, 35H, Ar), 5.42 [s, 1H, Ph$CH$(O–)$_2$], 5.19 (d, 1H, $J=$ 7.8 Hz, H-1a), 5.02 (d, 1H, $J=12.7$ Hz, $-CH_2Ph$), 4.81 (d, 1H, $J=11.0$ Hz, $-CH_2Ph$), 4.75–4.68 (m, 3H, $J=3$ Hz, $-CH_2Ph$), 4.60 (d, 1H, $J=12.5$ Hz, $-CH_2Ph$), 4.53 (d, 1H, $J=7.8$ Hz, H-1b), 4.48 (d, 1H, $J=12.2$ Hz, $-CH_2Ph$), 4.32–4.18 (m, 4H, H-2a, H-3a, H-6b, $-CH_2Ph$), 4.11 (brt, 1H, $J=8.9$ Hz, H-4a), 4.06 (brd, 1H, $J=3.4$ Hz, H-4b), 3.90 (brd, $J=11.0$ Hz, H-6b), 3.82–3.72 (m, 2H, H-6a, H-2b), 3.46 (dd, $J=3.4$, 9.4 Hz, H-3b), 3.36 (brd, 1H, $J=11.2$ Hz, H-6a),

3.16 (brs, 1H, H-5b), 3.12 (m, 1H, H-5a), 0.88 (s, 9H, *t*-Bu). Anal. calcd for $C_{71}H_{71}NO_{12}Si$: C, 73.61; H, 6.18; N, 1.21. Found: C, 73.61; H, 6.17; N, 1.21.

**5.1.8. *tert*-Butyldiphenylsilyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-2-amino-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside 14.** *Procedure A* (*reduction of* **12**). A mixture of **12** (324 mg, 0.31 mmol), powdered Zn (7 g) and AcOH (0.26 ml) in $CH_2Cl_2$ (50 ml) was stirred at room temperature for 3 h and then filtered through Celite. The filtrate was concentrated in vacuo and remaining AcOH was coevaporated with toluene. The residue was chromatographed on silica gel with $CHCl_3$ to give **14** (300 mg, 95%).

$[\alpha]_D=+4.9°$ (*c* 1). $R_f$ 0.06 ($CHCl_3$). $^1H$ NMR: $\delta$ 7.71–7.68 and 7.46–7.19 (m, 35H, Ar), 7.11–7.10 (m, 2H, N$H_2$), 5.45 [s, 1H, PhC*H*(O–)$_2$], 5.36 (d, 1H, *J*=10.7 Hz, –C$H_2$Ph), 4.77 (d, 1H, *J*=11.2 Hz, –C$H_2$Ph), 4.67 (brs, 2H, –C$H_2$Ph), 4.56 (d, 1H, *J*=10.7 Hz, –C$H_2$Ph), 4.50 (d, 1H, *J*=7.8 Hz, H-1b), 4.40 (d, 1H, *J*=12.0 Hz, –C$H_2$Ph), 4.37 (d, 1H, *J*=7.6 Hz, H-1a), 4.25 (d, 1H, *J*=12.2 Hz, –C$H_2$Ph), 4.07–4.03 (m, 2H, H-4a, H-6a), 3.90 (brd 1H, H-6b), 3.77–3.72 (m, 2H, H-6a, H-2b), 3.43 (dd, 1H, *J*=3.7, 9.5 Hz, H-3b), 3.30 (t, 1H, *J*=9.3 Hz, H-3a), 3.21 (br, 1H, H-4b), 3.09 (brs, 1H, H-5b), 3.00 (dd, 1H, *J*=7.6, 9.5 Hz, H-2a), 2.89 (br, 1H, H-5a), 1.08 (s, 9H, *t*-Bu). MALDI TOF MS: calcd for $C_{63}H_{69}NO_{10}Si\cdot Na$ *m/z* 1050.46. Found 1049.64. Anal. calcd for $C_{63}H_{69}NO_{10}Si$: C, 73.58; H, 6.76; N, 1.36. Found: C, 73.37; H, 6.80; N, 1.31.

*Procedure B* (*dephthaloylation of* **13**). A mixture of **13** (3.43 g, 2.97 mmol) and ethylenediamine (2.98 ml, 44.5 mmol) in *n*-BuOH (40 ml) was heated at 95°C for 18 h and then concentrated in vacuo. The residue was extracted with $CHCl_3$. The extract was successively washed with water and brine, dried over $Na_2SO_4$, and concentrated in vacuo. The crude product was chromatographed on silica gel with $CHCl_3$ to give **14** (2.99 g, 98%).

**5.1.9. *tert*-Butyldiphenylsilyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-trichloroacetamido β-D-glucopyranoside 15.** To a solution of **14** (95 mg, 0.09 mmol) in anhydrous pyridine (1.5 ml) was added trichloroacetyl chloride (80 μl, 0.28 mmol) at 0°C. The mixture was stirred at the temperature overnight and then diluted with ether. The ethereal extract was successively washed with water and brine, dried over $Na_2SO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel with hexane–EtOAc (3:1) to give **15** (94 mg, 87%). $[\alpha]_D=+4.6°$ (*c* 1). $R_f$ 0.58 (1:1 hexane–EtOAc). $^1H$ NMR: $\delta$ 7.69–7.62, 7.46–7.22, and 7.19–7.10 (m, 35H, Ar), 6.86 (d, 1H, *J*=7.3 Hz, N*H*), 5.44 [s, 1H, PhC*H*(O–)$_2$], 5.15 (d, 1H, *J*=10.7 Hz, –C$H_2$Ph), 4.86 (d, 1H, *J*=6.9 Hz, H-1a), 4.87–4.64 (m, 5H, *J*=5 Hz, –C$H_2$Ph), 4.50 (d, 1H, *J*=7.1 Hz, H-1b), 4.36 (d, 1H, *J*=12.0 Hz, –C$H_2$Ph), 4.20–4.08 (m, 3H, H-6a, H-6b, –C$H_2$Ph), 4.04 (br, 1H, H-4b), 3.89–3.81 (m, 3H, H-2a, H-6a, H-6b), 3.75–3.71 (m, 2H, H-3a, H-2b), 3.42 (dd, 1H, *J*=3.2, 9.5 Hz, H-3b), 3.20 (m, 1H, H-4a), 3.08 (brs, 1H, H-5b), 2.96 (m, 1H, H-5a), 1,05 (s, 9H, *t*-Bu). MALDI TOF MS: calcd for $C_{64}H_{68}Cl_3NO_{11}Si\cdot Na$ *m/z* 1194.36. Found 1194.27. Anal. calcd for $C_{64}H_{68}Cl_3NO_{11}Si\cdot 0.5H_2O$: C, 66.01; H, 5.88; N, 1.18. Found: C, 66.06; H, 5.88; N, 1.06.

**5.1.10. 2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-trichloroacetamido-D-glucopyranose 16.** To a mixture of **15** (236 mg, 0.20 mmol) and AcOH (127 ml, 2.01 mmol) in freshly distilled THF (6 ml) was added 1 M *n*-$Bu_4NF$/THF (0.8 ml, 0.80 mmol). The mixture was stirred at room temperature overnight and then concentrated in vacuo. The residue was extracted with EtOAc, successively washed with water and brine, dried over $Na_2SO_4$, and concentrated in vacuo. The crude product was chromatographed on silica gel with toluene–EtOAc (9:1) to give **16** (169 mg, 90%) as crystals. Mp 189–189.5°C. $R_f$ 0.34 (7:3 toluene–EtOAc). $^1H$ NMR: $\delta$ 7.45–7.18 (m, 25H, Ar), 6.79 (brd, 1H, N*H*), 5.44 [s, 1H, PhC*H*(O–)$_2$], 5.40 (t, 1H, *J*=3.7 Hz, H-1a), 5.21 (d, 1H, *J*=11.2 Hz, –C$H_2$Ph), 4.86 (d, 1H, *J*=11.0 Hz, –C$H_2$Ph), 4.77 (d, 1H, *J*=11.2 Hz, –C$H_2$Ph), 4.75–4.72 (m, 3H, *J*=3 Hz, –C$H_2$Ph), 4.54 (d, 1H, *J*=12.0 Hz, –C$H_2$Ph), 4.18 (m, 1H, H-6a), 4.12–4.04 (m, 3H, H-2a, H-4b, H-6b), 3.92 (dd, 1H, *J*=3.7, 10.5 Hz, H-6a), 3.98–3.82 (m, 3H, H-3a, H-4a, H-6b), 3.78 (brt, 1H, H-2b), 3.57 (m, 1H, H-5a), 3.22 (dd, *J*=3.4, 9.5 Hz, H-3b), 3.05 (m, 1H, H-5a), 3.00 (brs, 1H, O*H*). MALDI TOF MS: calcd for $C_{49}H_{50}Cl_3NO_{11}\cdot Na$ *m/z* 955.23. Found 955.74. Anal. calcd for $C_{49}H_{50}Cl_3NO_{11}$: C, 62.92; H, 5.39; N, 1.50. Found: C, 62.69; H, 5.39; N, 1.55.

**5.1.11. 2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-trichloroacetamido-α-D-glucopyranosyl fluoride 2.** To a stirred solution of **16** (114 mg, 0.12 mmol) in freshly distilled THF (3 ml) was added $Et_2NSF_3$ (28 μl, 0.17 mmol) at 0°C. The mixture was stirred for 30 min before the reaction was quenched with MeOH, and concentrated in vacuo. The residue was extracted with ether–EtOAc (1:1), successively washed with water and brine, dried over $Na_2SO_4$, and concentrated in vacuo. The crude product was chromatographed on silica gel with $CHCl_3$–EtOAc (9:1) to give **2** (113 mg, 93%). Mp 141.5–142°C (recrystallized from hexane–EtOAc). $[\alpha]_D=+38.3°$ (*c* 1.4). $R_f$ 0.68 (9:1 $CHCl_3$–EtOAc). $^1H$ NMR: $\delta$ 7.45–7.22 (m, 25H, Ar), 6.60 (d, 1H, *J*=7.3 Hz, N*H*), 5.77 (dd, 1H, *J*=2.5, 53.7 Hz, H-1a), 5.45 [s, 1H, PhC*H*(O–)$_2$], 5.21 (d, 1H, *J*=11.7 Hz, –C$H_2$Ph), 4.79 (d, 1H, *J*=11.5 Hz, –C$H_2$Ph), 4.78–4.74 (m, 3H, *J*=3 Hz, –C$H_2$Ph), 4.58 (d, 1H, *J*=12.0 Hz, –C$H_2$Ph), 4.41 (d, 1H, *J*=7.8 Hz, H-1b), 4.33 (d, 1H, *J*=12.0 Hz, –C$H_2$Ph). MALDI TOF MS: calcd for $C_{49}H_{49}Cl_3NO_{10}F\cdot Na$ *m/z* 958.22. Found 958.03. Anal. calcd for $C_{49}H_{49}Cl_3NO_{10}F$: C, 62.79; H, 5.27; N, 1.49; Cl, 11.35. Found: C, 62.76; H, 5.24; N, 1.45; Cl, 11.60.

**5.1.12. *N*-(9-Fluorenylmethoxycarbonyl)-*O*-{2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→3)]-2-azido-2-deoxy-α-D-galactopyranosyl}-L-serine allyl ester 17a.** A mixture of **3a** (144 mg, 0.13 mmol), $Cp_2ZrCl_2$ (71 mg, 0.24 mmol), $AgClO_4$ (100 mg, 0.48 mmol), and dried molecular sieves 4 Å (1.35 g) in anhydrous $CH_2Cl_2$ (8 ml) was stirred at −15°C under Ar for 1 h. Then a solution of **2** (113 mg, 0.12 mmol)

in anhydrous $CH_2Cl_2$ (3 ml) was added to the stirred mixture. The mixture was stirred at the temperature for 2.5 h, before the reaction was quenched with aq. $NaHCO_3$. The mixture was diluted with $CHCl_3$ and filtered through Celite. The filtrate was successively washed with sat. $NaHCO_3$, water, and brine, dried over $Na_2SO_4$, and concentrated in vacuo. The product was chromatographed on Bio-beads S X3 with toluene–EtOAc (1:1) to afford **17a** (184 mg, 76%). $[\alpha]_D=+34.4°$ (*c* 1). $R_f$ 0.23 (4:1 toluene–EtOAc). $^1$H NMR: δ 5.88 (m, 1H, $-CH_2CH{=}CH_2$), 5.72 (d, 1H, *J*=7.3 Hz, N*H*), 5.46 [s, 1H, PhC*H*$(O)_2$], 5.30 (brd, 1H, *J*=17.0 Hz, $-CH{=}CH_2$), 5.22 (brd, 1H, *J*=10.5 Hz, $-CH{=}CH_2$), 4.95 (d, 1H, *J*=9.0 Hz, GlcNTCA H-1), 4.87 (brd, 1H, *J*=3.4 Hz, $GalN_3$ H-1). $^{13}$C NMR: δ 98.0 ($^1J_{CH}$=170.7 Hz, $GalN_3$ C-1), 99.4 ($^1J_{CH}$=157.5 Hz, GlcNTCA C-1), 101.3 [Ph*C*H$(O)_2$], 102.7 ($^1J_{CH}$=160.9 Hz, Gal C-1), 103.9 ($^1J_{CH}$=155.9 Hz, Gal C-1). MALDI TOF MS: calcd for $C_{110}H_{112}Cl_3N_5O_{24}$·Na *m/z* 2014.67. Found 2014.42. Anal. calcd for $C_{110}H_{112}Cl_3N_5O_{24}$: C, 66.24; H, 5.66; N, 3.51; Cl, 5.33. Found: C, 66.20; H, 5.81; N, 3.29; Cl, 5.63.

**5.1.13. *N*-(9-Fluorenylmethoxycarbonyl)-*O*-{2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzyl-2-deoxy-2-trichloroacetamido-β-D-glucopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→3)]-2-azido-2-deoxy-α-D-galactopyranosyl}-L-threonine allyl ester 16b.** Condensation of **3b** (146 mg, 0.13 mmol) and **2** (115 mg, 0.12 mmol) was performed in the same procedure as described for **16a**. Chromatography of the crude product on Bio-beads S X3 gave **16b** (172 mg, 70%). $[\alpha]_D=+33.8°$ (*c* 1). $R_f$ 0.30 (4:1 toluene–EtOAc). $^1$H NMR: δ 5.91 (m, 1H, $-CH_2CH{=}CH_2$), 5.67 (d, 1H, *J*=9.3 Hz, N*H*), 5.44 [s, 1H, PhC*H*$(O)_2$], 5.33 (brd, 1H, *J*=17.0 Hz, $-CH{=}CH_2$), 5.23 (brd, 1H, *J*=10.3 Hz, $-CH{=}CH_2$), 5.19 (d, 1H, *J*=10.2 Hz, N*H*), 4.95 (d, 1H, *J*=3.2 Hz, $GalN_3$ H-1), 4.87 (d, 1H, *J*=7.9 Hz, GlcNTCA H-1), 1.31 (d, 1H, *J*=6.1 Hz, Thr γH). $^{13}$C NMR: δ 99.6 ($^1J_{CH}$=172.5 Hz, $GalN_3$ C-1), 99.8 ($^1J_{CH}$=159.2 Hz, GlcNTCA C-1), 101.2 [Ph*C*H$(O)_2$], 102.6 ($^1J_{CH}$=160.0 Hz, Gal C-1), 103.8 ($^1J_{CH}$=162.5 Hz, Gal C-1). MALDI TOF MS: calcd for $C_{111}H_{114}Cl_3N_5O_{24}$·Na *m/z* 2028.69. Found 2028.41. Anal. calcd for $C_{111}H_{114}Cl_3N_5O_{24}$·$H_2O$: C, 65.78; H, 5.77; N, 3.46. Found: C, 65.89; H, 5.78; N, 3.28.

**5.1.14. *N*-(9-Fluorenylmethoxycarbonyl)-*O*-{2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→3)]-2-acetamido-2-deoxy-α-D-galactopyranosyl}-L-serine allyl ester 18a.** A mixture of **17a** (142 mg, 0.07 mmol), AcOH (1.5 ml), and Zn powder (1.5 g) in $CH_2Cl_2$ (35 ml) was stirred at room temperature for 72 h, and then filtered through Celite. The filtrate was concentrated in vacuo to the residue, which was dissolved in MeOH–$CH_2Cl_2$ (1:1, 1 ml) and stirred with $Ac_2O$ (36 μl, 0.36 mmol) for 40 min. The mixture was diluted with $CH_2Cl_2$ and successively washed with sat. $NaHCO_3$, water, and brine, dried over $Na_2SO_4$, and concentrated in vacuo. The residue was chromatographed on Sephadex LH-60 with $CHCl_3$–MeOH (1:1) to give **18a** (130 mg, 92%). $[\alpha]_D=+31.5°$ (*c* 1). $R_f$ 0.23 (1:4 toluene–EtOAc). $^1$H NMR: δ 6.34 (d, 1H, *J*=7.3 Hz, N*H*), 5.94–5.83 (m, 2H, N*H*, $-CH_2CH{=}CH_2$), 5.56 (d, 1H, *J*=9.2 Hz, N*H*), 5.43 [s, 1H, PhC*H*$(O)_2$], 5.30 (brd, 1H, *J*=17.1 Hz, $-CH{=}CH_2$), 5.20 (brd, 1H, *J*=10.5 Hz, $-CH{=}CH_2$), 1.80 (s, 3H, Ac), 1.55 (s, 3H, Ac). $^{13}$C NMR: δ 98.0 ($^1J_{CH}$=172.5 Hz, GalNAc C-1), 100.3 ($^1J_{CH}$=165.9 Hz, GlcNAc C-1), 101.2 [Ph*C*H$(O)_2$], 102.8 ($^1J_{CH}$=163.4 Hz, Gal C-1), 104.2 ($^1J_{CH}$=161.7 Hz, Gal C-1). MALDI TOF MS: calcd for $C_{112}H_{119}N_3O_{25}$·Na *m/z* 1928.81. Found 1928.84. Anal. calcd for $C_{112}H_{119}N_3O_{25}$: C, 70.53; H, 6.29; N, 2.20. Found: C, 70.55; H, 6.22; N, 2.39.

**5.1.15. *N*-(9-Fluorenylmethoxycarbonyl)-*O*-{2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→3)]-2-acetamido-2-deoxy-α-D-galactopyranosyl}-L-threonine allyl ester 18b.** Compound **17b** (86 mg, 0.04 mmol) was treated with Zn and AcOH in $CH_2Cl_2$, and then the reduced product was acetylated in $CH_2Cl_2$–MeOH as described above for **18a**. The crude product was purified by gel-permeation chromatography on Sephadex LH-60 to afford **18b** (80 mg, 96%). $[\alpha]_D=+36.7°$ (*c* 1). $R_f$ 0.37 (1:4 toluene–EtOAc). $^1$H NMR: δ 5.84 (m, 1H, $-CH_2CH{=}CH_2$), 5.67 (d, 1H, *J*=9.0 Hz, N*H*), 5.62 (d, 1H, *J*=8.5 Hz, N*H*), 5.58 (d, 1H, *J*=8.1 Hz, N*H*), 5.43 [s, 1H, PhC*H*$(O)_2$], 5.30 (brd, 1H, *J*=17.6 Hz, $-CH{=}CH_2$), 5.24 (brd, 1H, *J*=10.3 Hz, $-CH{=}CH_2$), 1.81 (s, 3H, Ac), 1.65 (s, 3H, Ac), 1.29 (d, 1H, *J*=6.0 Hz, Thr γH). $^{13}$C NMR: δ 99.5 ($^1J_{CH}$=178.3 Hz, GalNAc C-1), 100.7 ($^1J_{CH}$=164.2 Hz, GlcNAc C-1), 101.1 [Ph*C*H$(O)_2$], 103.5 ($^1J_{CH}$=159.2 Hz, Gal C-1), 104.3 ($^1J_{CH}$=159.2 Hz, Gal C-1). MALDI TOF MS: calcd for $C_{113}H_{121}N_3O_{25}$·Na *m/z* 1942.83. Found 1943.17. Anal. calcd for $C_{113}H_{121}N_3O_{25}$: C, 70.64; H, 6.35; N, 2.19. Found: C, 70.38; H, 6.67; N, 2.05.

**5.1.16. *N*-(9-Fluorenylmethoxycarbonyl)-*O*-{2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→3)]-2-acetamido-2-deoxy-α-D-galactopyranosyl}-L-serine 1a.** To a mixture of **18a** (61 mg, 0.03 mmol), 5,5-dimethyl-1,3-cyclohexanedione (dimedone, 89 mg, 0.6 mmol), and $Pd(PPh_3)_4$ (8 mg, mmol) was added freshly distilled THF (30 ml) under Ar. The mixture was stirred overnight at room temperature and then concentrated in vacuo. The residue was chromatographed on silica gel with ($CHCl_3$–EtOH (14:1, 1% AcOH) and then on Sephadex LH-60 with $CHCl_3$–MeOH (1:1) to give **1a** (57 mg, 97%). $[\alpha]_D=+28.1°$ (*c* 1). $R_f$ 0.15 (14:1 $CHCl_3$–EtOH, 1% AcOH). $^1$H NMR (DMSO-*d*6): δ 5.63 [s, 1H, PhC*H*$(O)_2$], 1.78 (s, 3H, Ac), 1.64 (s, 3H, Ac). HR Fab MS: calcd for $C_{109}H_{115}N_3O_{25}$·Na *m/z* 1889.7751. Found 1889.7744.

**5.1.17. *N*-(9-Fluorenylmethoxycarbonyl)-*O*-{2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl-(1→3)]-2-acetamido-2-deoxy-α-D-galactopyranosyl}-L-threonine 1b.** As described for **1a**, compound **18b** (114 mg, 0.06 mmol) was deallylated with dimedone (165 mg, 1.18 mmol) and $Pd(PPh_3)_4$ (14 mg, 0.01 mmol), and the product was purified by chromatography to give **1b** (104 mg, 93%). $[\alpha]_D=+34.4°$ (*c* 1). $R_f$ 0.08 (14:1

$CHCl_3$–EtOH, 1% AcOH). $^1$H NMR (DMSO-*d*6): δ 5.63 [s, 1H, PhC*H*(O)$_2$], 1.78 (s, 3H, Ac), 1.67 (s, 3H, Ac), 1.10 (d, 3H, *J*=5.6 Hz, Thr γH). $^{13}$C NMR ($CDCl_3$): δ 98.9 (GalNAc C-1), 100.8 (GlcNAc C-1), 101.1[Ph*C*H(O)$_2$], 102.8 (Gal C-1), 104.3 (Gal C-1).

HR Fab MS: calcd for $C_{110}H_{117}N_3O_{25}$·Na *m/z* 1902.7874. Found 1902.7854.

**5.1.18. *N*-(9-Fluorenylmethoxycarbonyl)-*O*-{β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→3)]-2-acetamido-2-deoxy-α-D-galactopyranosyl}-L-threonine 19.** A mixture of **1b** (1 mg), dimethylsulfide (3 μl), *m*-cresol (1 μl) and TFA (5 μl) in a polypropylen microcentrifuge tube was cooled at −15°C with stirring. TfOH (1 μl) was added to the mixture, which was stirred for 1 h. Then a precooled (−80°C) solution of pyridine (200 μl) in ether (1 ml) was added and the mixture was vigorously stirred for 1 min with a vortex mixer. The precipitate was separated by centrifugation, the ethereal layer was decanted, and the residual precipitate was washed two times with ether by vortex-mixing, centrifugation and decantation. The product thus obtained was dissolved in 20% aq. $CH_3CN$ and analyzed by HPLC. (for chromatographic conditions see Fig. 3(a))

**5.1.19. *N*-(9-Fluorenylmethoxycarbonyl)-L-Prolyl-*O*-{β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→3)]-2-acetamido-2-deoxy-α-D-galactopyranosyl}-L-threonyl-L-threonyl-L-seryl-L-threonyl-L-asparaginyl-L-alanyl-L-seryl-L-threonyl-L-valylamide 20.** All the solid-phase reactions were performed in a polylpropylene tube equipped with a coarse filter and three-way stopcock by stirring with a vortex mixer. Commercial Sieber amide resin (403 mg, 0.25 mmol) was stirred with 20% piperidine/NMP (5 ml) for 5 min. After filtration the resin was again stirred with 20% piperidine/NMP (5 ml) for 15 min to complete *N*-deprotection. The resin was washed several times with NMP (5 ml) and then stirred with Fmoc-Val-OH (339 mg, 1 mmol), 1 M DCC/NMP (1 ml, 1 mmol), 1 M HOBt/NMP (1 ml, 1 mmol) in NMP (5 ml) for 1 h. The mixture was filtered and the resultant resin was washed successively with NMP and MeOH–$CH_2Cl_2$ (1:1). The unreacted amino group on the resin was acetylated with 10% $Ac_2O$–5% DIEA/NMP (5 ml). After washing with NMP, the resin was *N*-deprotected in the same manner as described above, and used for further peptide assembling with Fmoc-Thr(Bu$^t$)-OH, Fmoc-Ser(Bu$^t$)-OH, Fmoc-Ala-OH, Fmoc-Asn(Trt)-OH, Fmoc-Thr(Bu$^t$)-OH, Fmoc-Ser(Bu$^t$)-OH, Fmoc-Thr(Bu$^t$)-OH. Fmoc amino acids (4 equiv.), DCC (4 equiv.), and HOBt (4 equiv.) were used for each coupling reaction. *N*-Deprotection and acetyl capping reaction were performed as described. A part of the octapeptide-resin thus prepared was subjected to the condensation with **1b**. The *N*-deprotected resin (50 mg, 20 μmol) was stirred with **1b** (75 mg, 40 μmol), HATU (14 mg, 38 μmol), and DIEA (8 μl, 45 μmol) in NMP (0.6 ml) overnight.

The resin was washed with NMP and MeOH–$CH_2Cl_2$, then *N*-deprotected, and condensed with Fmoc-Pro-OH to complete the sequence.

*Cleavage and debenzylation under the conditions of low acidity TfOH*. A mixture of the resin (1 mg), dimethylsulfide (3 μl), *m*-cresol (0.8 μl), 1,2-ethanedithiol (0.2 μl) and TFA (5 μl) in a polypropylen microcentrifuge tube was cooled at −15°C with stirring. TfOH (1 μl) was added to the mixture, which was stirred for 1 h. The reaction was quenched with ethereal pyridine and the work-up procedure was done as described for **19**. The product was analyzed by HPLC and MALDI TOF MS (Fig. 3(b)).

Peak 1 (**20**): calcd for $C_{82}H_{124}N_{14}O_{39}$·Na *m/z* 1951.81. Found 1951.45. Peak 2: $C_{49}H_{68}N_{10}O_{14}$·Na *m/z* 1043.48. Found 1042.97. Peak 3: $C_{101}H_{138}N_{14}O_{39}$·Na *m/z* 2193.91. Found 2193.57.

*Cleavage and debenzylation under the conditions involving reagent K*. The resin (1 mg) was stirred with reagent K [$CF_3CO_2H$–phenol–deionized water–thioanisole–ethanedithiol (82.5:5:5:5:2.5), 10 μl] for 1 h. The volatile components in the mixture were removed by blowing nitrogen. Ether was added to the residue, and the mixture was centrifuged. The ether layer was decanted, and the precipitate was again washed with ether. The precipitated mixture of glycopeptide and resin was subjected to the procedure for the low acidity TfOH as described above. The operation for the debenzylation procedure was performed for either 1 or 2 h. Figure 3(c) shows chromatogram of the products via the 2 h operation. MALDI TOF MS: Peak 1: calcd for $C_{30}H_{55}N_{10}O_{14}$ *m/z* 779.39. Found 778.62. Peak 2 (**20**): found 1951.31. Peak 3: calcd for $C_{76}H_{114}N_{14}O_{34}$·Na *m/z* 1789.75. Found 1789.73. Peak 4: found 1789.68. Peak 5: calcd for $C_{68}H_{101}N_{13}O_{29}$·Na *m/z* 1586.67. Found 586.77. Peak 6: calcd for $C_{50}H_{71}N_{11}O_{17}$·Na *m/z* 1120.49. Found 1120.83.

**5.1.20. L-Prolyl-*O*-{β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→3)]-2-acetamido-2-deoxy-α-D-galactopyranosyl}-L-threonyl-L-threonyl-L-seryl-L-threonyl-L-asparaginyl-L-alanyl-L-seryl-L-threonyl-L-valylamide 21.** The de-*N*-Fmoc resin was prepared from the above glycopeptide-resin by the routine treatment with 20% piperidine. The resulting resin (11 mg) was treated with reagent K (110 μl), and the cleaved glycopeptide was debenzylated at −15°C for 2 h with dimethylsulfide (12 μl), *m*-cresol (3.2 μl), 12-ethanedithiol (0.8 μl), TFA (20 μl) and TfOH (4 μl) as described for **20**. The product was purified by HPLC (Fig. 3(d)), the collected fractions were concentrated and then lyophilized to give **21** (1.6 mg, 27%). MALDI TOF MS: calcd for $C_{67}H_{115}N_{14}O_{37}$·Na *m/z* 1707.74. Found 1707.70; for $C_{67}H_{114}N_{14}O_{37}$·Na *m/z* 1729.74. Found 1729.65.

Amino acid analysis: Asp/Asn$_{1.01}$Thr$_{3.55}$Ser$_{1.68}$Pro$_{0.96}$ Ala$_{1.00}$Val$_{1.24}$.

**5.1.21. *N*-(9-Fluorenylmethoxycarbonyl)-*O*-{(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→3)]-2-acetamido-2-deoxy-α-D-galactopyranosyl}-L-threonine 22.** To a solution of **19** (100 nmol) in deionized water (10 μl) were added 0.5 M 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer (pH 6.8, 39 μl),

bovine serum albumin (BSA) in MOPS buffer (5 mg/ml; 5 μl), 0.1 M CMP–sialic acid (5 μl), and recombinant rat β-Gal-β1,3/4-GlcNAc-α2,3-sialyltransferase (1 μl, 3.7 mU). The mixture was incubated at 37°C, and analyzed by HPLC in combination with MALDI TOF MS. The chromatogram for the mixture of 24 h incubation is depicted in Figure 5(a).

**5.1.22. *N*-(9-Fluorenylmethoxycarbonyl)-*O*-{β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-[(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→3)]-2-acetamido-2-deoxy-α-D-galactopyranosyl}-L-threonine 23.** A solution of **19** (100 nmol) in deionized water (10 μl) was incubated with 0.1 M CMP–sialic acid (5 μl), recombinant rat β-Gal-β1,3-GalNAc-α2,3-sialyltransferase (1 μl, 0.9 mU), BSA solution (5 μl) and 0.5 M MOPS buffer (pH 6.0, 29 μl) at 37°C. The chromatogram for the reaction mixture of 16 h incubation is shown in Figure 5(b).

**5.1.23. *N*-(9-Fluorenylmethoxycarbonyl)-*O*-{(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-[(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→3)]-2-acetamido-2-deoxy-α-D-galactopyranosyl}-L-threonine 24.** A solution of **19** (100 nmol) in deionized water (10 μl) was incubated with 0.1 M CMP–sialic acid (15 μl), recombinant rat β-Gal-β1,3/4-GlcNAc-α2,3-sialyltransferase (1 μl, 3.7 mU), recombinant rat β-Gal-β1,3-GalNAc-α2,3-sialyltransferase (1 μl, 0.9 mU), BSA solution (5 μl) and 0.5 M MOPS buffer (pH 6.8, 18 μl) at 37°C. The chromatogram for the reaction mixture of 12 h incubation is shown in Figure 5(c).

**5.1.24. L-Prolyl-*O*-{(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-[(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→3)]-2-acetamido-2-deoxy-α-D-galactopyranosyl}-L-threonyl-L-threonyl-L-seryl-L-threonyl-L-asparaginyl-L-alanyl-L-seryl-L-threonyl-L-valylamide 25.** Glycopeptide **21** (100 nmol) was sialylated under the same conditions as described for **24**. The mixture was dissolved in 10 mM ammonium acetate containing 2% acetonitrile and analyzed by HPLC eluted with 10 mM ammonium acetate buffer–acetonitrile combination as shown in Figure 6. MALDI TOF MS: calcd for $C_{89}H_{148}N_{16}O_{53}(+H^+)$ *m/z* 2289.93. Found 2289.82; calcd for $C_{89}H_{148}N_{16}O_{53}\cdot Na$ *m/z* 2311.93. Found 2311.35.

**5.1.25. L-Prolyl-*O*-{(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→3)]-2-acetamido-2-deoxy-α-D-galactopyranosyl}-L-threonyl-L-threonyl-L-seryl-L-threonyl-L-asparaginyl-L-alanyl-L-seryl-L-threonyl-L-valylamide 26.** Glycopeptide **21** (100 nmol) was sialylated and isolated in a similar manner as described for **25**. MALDI TOF MS: calcd for $C_{78}H_{131}N_{15}O_{45}(+H^+)$ *m/z* 1998.85. Found 1998.52; calcd for $C_{78}H_{131}N_{15}O_{45}\cdot Na$ *m/z* 2020.83. Found 2020.67.

**5.1.26. L-Prolyl-*O*-{β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→6)-[(5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2→3)-β-D-galactopyranosyl-(1→3)]-2-acetamido-2-deoxy-α-D-galactopyranosyl}-L-threonyl-L-threonyl-L-seryl-L-threonyl-L-asparaginyl-L-alanyl-L-seryl-L-threonyl-L-valylamide 27.** Glycopeptide **21** (100 nmol) was sialylated and isolated in a similar manner as described for **25**. MALDI TOF MS: calcd for $C_{78}H_{131}N_{15}O_{45}(+H^+)$ *m/z* 1998.85. Found 1998.56; calcd for $C_{78}H_{131}N_{15}O_{45}\cdot Na$ *m/z* 2020.83. Found 2020.60.

## Acknowledgements

We are indebted to Dr T. Chihara and his staff (Riken) for the combustion analyses. This work was supported by Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan [(B) No.13460051] and in part by CREST program of Japan Science and Technology Corporation. The authors also acknowledge Tokai University for a grant-aid for high-technology research.

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